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PROVISIONAL SPECIFICATION

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Invention Title: **Xenotransplantation therapies.**

The invention is described in the following statement:

5014

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TITLE: *Xenotransplantation Therapies*

This invention relates to xenotransplantation (transplantation across species) and is particularly concerned with methods of alleviating xenotransplant rejection, maintenance of xenotransplanted tissue in an animal, nucleotide sequences useful in xenotransplant therapies, rejection resistant transgenic organs, and transgenic animals whose tissues are rejection-resistant on xenotransplantation.

The current shortage of tissues for human transplantation has led to recent close examination of xenografts as a possible source of organs. However, when tissues from non-human species are grafted to humans, hyperacute rejection occurs due to the existence of natural antibodies in human serum which react with antigens present in these species, with rejection occurring within 10-15 minutes of transplantation. This phenomenon depends, in general, on the presence of some or all of antibody, complement, neutrophils, platelets and other mediators of inflammation. In transplantation of vascularized organs between "discordant" species (those in which natural antibodies occur) the first cells to encounter natural antibodies are the endothelial cells lining the blood vessels and it is likely that activation of these cells is induced by antibody binding to xenoantigens or other factors, leading to hyperactue rejection.

There is considerable uncertainty in the art concerning the nature of possible target xenoantigens on xenograft tissues, Platt et al (Transplantation 50:817-822,1990) and Yang et al (Transplant. Proc.24:593-594, 1992) have identified a triad of glycoproteins of varying molecular weights as the major targets on the surface of pig endothelial cells. Other investigators (Holgersson et al, Trasplant. Proc. 24:605-608, 1992) indicate glycolipids as key xenoantigens.

We have now found that xenograft rejection, particularly in the context of pig tissue, is associated with antibodies reactive with galactose in an $\alpha(1,3)$ linkage with galactose, (the Gal$\alpha(1,3)$Gal epitope). Modulating the interaction between antibodies reactive with the Gal$\alpha(1,3)$Gal epitope of xenotransplant tissue effects rejection.

In accordance with the first aspect of this invention, there is provided a method of inhibiting xenotransplant rejection in an animal patient, comprising administering to the patient an effective amount of an antagonist of antibody binding to xenotransplant antigens having galactose in an $\alpha(1,3)$ linkage with galactose.

Another aspect of this invention relates to the maintenance of xenograft tissue in an animal, which comprising administering to the animal a graft rejection effective amount of an antagonist to antibodies which bind to the xenograft antigen epitope Gal$\alpha(1,3)$Gal.

In another aspect of this invention there is provided a method of inhibiting the binding of antibodies to the Gal$\alpha(1,3)$Gal epitope which comprises modulating the interaction between the antibodies and the epitope with an antagonist which blocks the binding of the antibodies to the Gal$\alpha(1,3)$Gal epitope.

Preferably the xenograft recipient is a human. Age is not a determining factor for xenograft transplantation although transplants in the elderly over 75 years would normally not be carried out. The invention is directed particularly to human transplantation with xenograft tissue.

Xenografted tissue is preferably of pig origin. Tissues from other mammals are also contemplated for use in this use invention. Preferably the xenotransplanted tissue is in the form of an organ, for example, kidney, heart, lung or liver. Xenotransplant tissue may also be in the form of parts of organs, cell clusters, glands and the like. Examples include lenses, pancreatic islet cells, skin and corneal tissue. The nature of the xenotransplanted tissue is not of itself critical as any xenotransplanted tissue which expresses antigens having Gal$\alpha(1,3)$Gal epitopes may be utilized in accordance with this invention.

The binding of antibody to the Gal$\alpha(1,3)$Gal epitope expressed on xenotransplanted tissue provokes rejection of the tissue by humoral as well as cell-mediated immune effects

leading to tissue rejection in a very short time scale, such as less than one hour. Antagonists which antagonize the binding of antibodies to the Galα(1,3)Gal epitope block antibody binding and therefore inhibit xenotransplant rejection. Because antibody binding is blocked, immune response which gives rise to tissue rejection are prevented.

In accordance with a further aspect of this invention, there is provided an antagonist which modulates the interaction of antibodies directed against Galα(1,3)Gal.

Any antagonist capable of modulating the interaction between antibodies directed to the Galα(1,3)Gal linkage may utilized in this invention. By reference to modulation, is meant blockage of antibody binding or decrease in affinity reactivity of antibodies for the Galα(1,3)Gal epitope. Various mechanisms may be associated with the blockage of antibody binding or decreased affinity of antibodies for their respective epitope. These include binding or association with the antibody reactive site and change of conformation of the antibody reactive site, (such as by binding to residues associated with adjacent to or distanced from the active site (which effect the conformation of the active site such that it is incapable of binding the Galα(1,3)Gal epitope or binds the epitope with reduced affinity). This invention is not limited to any specific antagonist and any antagonist which is non-toxic and which modulates the interaction between antibodies specific for the Galα(1,3)Gal epitope may be used in this invention. Suitable examples of antagonists include D-galactose and melibiose, stachose and methyl-α-D-galactopyranoside, D-galactosamine and derivatives thereof. The term derivatives encompasses, for example, any alkyl, alkoxy, alkylkoxy, aralkyl amine, hydroxyl, nitro, hetrocycle, sulphate and/or cycloalkyl substituents whether taken alone or in combination, which derivates have antagonist activities. This may be assessed according to methods as herein described. Carbohydrate polymers containing one or more of the aforesaid carbohydrate moities or derivaties may also be utilized in this invention.

The amount of antagonists which is effective to modulate interaction between antibodies reactive with Galα(1,3)Gal epitopes will vary depending upon a number of factors.

These include the nature of the animal being treated, the nature of species of the transplanted tissue, the physical condition of the transplant recipient (age, weight, sex and health) and the like. In respect of human transplant recipients of tissue, for example from pigs, the amount of antagonists administered will generally depend upon the judgement of a consulting physician. As an example, a graft rejection effective amount of an antagonist in human subjects may be in the order of from 0.01mg to 1000gm per dose, more preferably 10mg to 500mg, more preferably 50mg to 300mg, and still more preferably 50mg to 200mg per dose.

The schedule of administration of antagonists to inhibit rejection and maintain xenografts will depend upon varying factors as mentioned above. Varying dosage regimes may be contemplated, such as daily, weekly, monthly or the like.

The mode of administration of antagonists and dosage forms thereof are not critical to this invention. Antagonists may be administered parenterally (intravenous, intramuscular or intraorgan injection), orally, transdermally, or by vaginal or anal routes, or by other routes of administration, as are well known in the art. Antagonists may be in solid or liquid form and would generally include pharmaceutically acceptable or veterinarially acceptable excipients and/or carriers. Examples of dosage forms which may be used in this invention are those well known in the art as mentioned previously such as described in Remington's Pharmaceutical Sciences (Mack Publishing Company, 10th Edition, which is incorporated herein by reference).

In still another aspect of this invention, there is provided nucleotide sequences encoding $\alpha(1,3)$galactosyl transferase and mutants thereof. Preferably, the nucleotide sequence encodes pig $\alpha(1,3)$ galactosyl transerase.

Nucleotide sequences may be in the form of DNA, RNA or mixtures thereof. Nucleotide sequences or isolated nucleic acids may be inserted into replicating DNA, RNA or DNA/RNA vectors as are well known in the art, such as plasmids, viral vectors, and the like (Sambrook et al, Molecular Cloning A Laboratory Manual, Cold Spring Harbor

Laborartory Press, NY, Second Edition 1989).

Nucleotide sequences encoding $\alpha(1,3)$galactosyl transferase may include promoters, enhancers and other regulatory sequences necessary for expression, transcription and translation. Vectors encoding such sequences may include restriction enzyme sites for the insertion of additional genes and/or selection markers, as well as elements necessary for propagation and maintenance of vectors within cells.

Mutants of nucleotide sequences encoding $\alpha(1,3)$galactosyl transferase are particularly preferred as they may be used in homologous recombination techniques as are well known in the art (Capecchi M R, Altering the Genome by Homologous Recombination, Science 244:1288-1292, 1989; Merlino G T, Transgenic Animals in Biomedical research, FASEB J 5:2996-3001, 1991; Cosgrove et al, Mice Lacking MHC Class II Molecules, Cell 66:1051-1066, 1991; Zijlstra et al, Germ-line Transmission of a disrupted B2-microglobulin gene produced by homologous recombination in embryonic stem cells Nature 342:435-, 1989) for the inactivation of wild type $\alpha(1,3)$galactosyl transferase genes.

Mutant $\alpha(1,3)$galactosyl transferase nucleotide sequences include nucleotide deletions, insertions, substitutions and additions to wild type $\alpha(1,3)$galactosyl transferase such that the resultant mutant does not encode a functional galactosyl transferase. These nucleotide sequences may be utilized in homologous recombination techniques. In such techniques, mutant sequences are recombined with wild type genomic sequences in stem cells, ova or newly fertilized cells comprising from 1 to about 500 cells. Nucleotide sequences utilized in homologous recombination may be in the form of isolated nucleic acids sequences or in the context of vectors. Recombination is a random event and on recombination, destruction of the functional gene takes place.

Transgenic animals produced by homologous recombination and other such techniques to destroy wild type gene function are included within this invention, as are organs derived

therefrom. By way of example, transgenic pigs may be produced utilizing homologous recombination techniques to produce a transgenic animal having non-functional α(1-3)galactosyl transferase genomic sequences. Tissues derived from such transgenic animals may then be utilized in xenotransplantation into human patients with the avoidance of immune reaction between circulating human antibodies reactive with Galα(1-3)Gal epitopes. Such transplants are contemplated to be well tolerated by transplant recipients. Whilst transplanted tissue may comprise other antigens which provoke immune reaction beyond those associated with Galα(1-3)Gal epitopes, removing the major source of the immune reaction with such transplanted tissues should lead to xenotransplants being relatively well tolerated in conjunction with standard rejection therapy (treatment with immune supressants such as cyclosporin).

This invention will now be described with reference to the following non-limiting Figures and Examples.

FIGURE LEGENDS:

Figure 1: Titer of pooled human serum before and after absorption. Titer obtained by hemagglutination on RBC (▨) and rosetting assay on PBL (□) and spleen cells (■). Absorption studies demonstrated that the same xeno antigens were present on all of these tissues (Figure 1 and Figure 2), as absorption with RBC, spleen cells or PBL, removed reactivitiy for the other cells (Figure 1A and Figure 2. absorption of the serum pool with EC, while removing all of the EC reactive antibodies (Figure 2a), completely removed all PBL reactive antibodies and almost all the RBC hemagglutinating antibodies (titer fell from 1/128 to 1/2) (Figure 1A). Absorption with RBC removed 75% (Figure 2b) and spleen cells all (Figure 2c) of the EC reactive antibodies shown by flow cytometry. Thus, common epitopes are present on pig red cells, PBL< spleen and endothelial cells. Serum absorbed with EC was not tested on PBL or spleen

cells and is noted in the figure with an *.

Figure 2: Testing of pig EC with pooled human serum before and after absorption. In each panel EC tested with absorbed serum (.....) or non abosrbed serum (___). Serum absorbed with EC (panel A), RBC (panel B) or spleen cells (panel C). Binding of human antibody was detected using sheep anti-human IgM and analysis by flow cytometry.

Figure 3: Hemagglutination titer of treated and untreated human serum. Untreated human serum (A); protein-A non binding immunoglobulin (B) and protein-A eluted immunoglobulin (C); serum treated with 2-mercaptoethanol (D).

Figure 4: Carbohydrate inhibition of hemagglutination of normal human serum. Human serum was titered in the presence of 300mM solutions of carbohydrates.

Figure 5: Concentration of carbohydrate giving 50% inhibition of hemaggultination titer of normal human serum. Only carbohydrates inhibiting hemagglutination in Figure 4 were used in this experiment, with glucose and methyl-β-galactopyranoside as negative controls.

Figure 6: Hemagglutination titer of human serum on pig RBC pre and post absorption on a melibiose column. Human serum was absorbed with equal volumes of melibiose-sepharose (■) or sepharose (□), a number of times as indicated in the figure axis.

Figure 7: Nucleotide and predicted amino acid sequence of the pig Galα(1,3) transferase

EXAMPLE 1

Materials and Methods

Cells. Pig cells and tissues were obtained from an abattoir from freshly slaughtered animals. Whole blood was centrifuged at 800g, and erythrocytes (RBC) obtained and were washed three times in phosphate buffered saline (PBS); pig peripheral blood lymphocytes (PBL) were isolated by density gradient centrifugation using isopaque ficoll (Vaughan et al, (1983) Tansplantaton 36:446-450). Pig splenocytes were obtained from whole spleen by teasing tissue through a sieve to give a single cell suspension. Endothelial cell (EC) cultures were established after treatment of sterile pig aorta with Collagenase Type 4 (Worthington Biochemical Corporation, New Jersey) and the isolated cells were grown in Dulbeccos modified Eagles medium (DMEM) (ICN Biomedicals Australasia Pty Ltd, Seven Hills, NSW) on gelatin coated plates at 37°C. The endothelial origin of EC cultures was verified using rabbit anti human von Willibrand factor antibody (Dako A/S, Copenhagen) and indirect immunofluorescence. COS cells used were maintained in fully supplemented DMEM medium.

Antibodies. Human serum was obtained from a panel of normal volunteers, heat inactivated and pooled before use. The mAb HuLy-m3 (CD48), was used as a negative control (Vaughan Supra). Equal volumes of human serum and 5 to 200mM 2-mercaptoethanol were incubated at 37°C for one hour to destroy IgM.

Absorptions. Pooled serum was absorbed with equal volumes of washed, packed cells for 15 minutes at 37°C, for 15 minutes at 4°C, serum obtained and the procedure repeated three times. For the absorption with melibiose-agarose (Sigma, St Louis, MO) and sepharose (Pharmacia LKB Biotechnology, Uppsala, Sweden), equal volumes packed beads and serum were incubated at 37°C for 16 hours, the beads removed by centrifugation, and the absorption repeated several times.

Serological Assays. a) Hemagglutination: 50μl of 0.1% pig RBC were added to 50μl of human serum in 96 well plates, incubated at 37°C for 30 minutes, room temperature for 30 minutes and on ice for 60 minutes prior to both macroscopic and microscopic evaluation of hemagglutination; b) Rosetting: Sheep anti human IgG was coupled to sheep RBC with chromic chloride and used in a rosetting assay (Parish et al (1978) J Immunol. Methods 20:173-183); c) Cytofluorographic analysis was performed on FACScan (Becton Dickinson, San Jose, CA) (Vaughan et al (1991) Immunogenetics 33:113-117); d) Indirect immunofluorescence was performed on cell monolayers in 6 well tissue culture plates using fluorescienated sheep anti human IgM or IgG (Silenus Laboratories Pty Ltd, Hawthorn, Victoria, Australia) (Vaughan Supra).

Sugar Inhibitions. Two types of sugar inhibition assays were performed: a) 50μl of sugars (300mM in PBS) were added to 50μl of doubling dilutions of human serum in 96 well plates, incubated overnight at 4°C and then 50μl of 0.1% pig RBC added and the hemagglutination assay performed; b) Human serum, diluted in PBS at one dilution less than that of the 50% hemagglutination titer, was added to 50μl of doubling dilutions os sugars (starting at 300mM) and incubated overnight at 4°C, after which 50μl of 0.1% pig RBC added and the hemagglutination assay performed.

Murine gal α(1-3)transferase cDNA construct. A cDNA clone, encoding the mouse α(1,3)galactosyl transferase was produced using the published sequence of this transferase (Larsen et al (1989) J Biol. Chem 264:14290-14297) and the polymerase chain reaction (PCR) technique. Briefly two oligonucleotides were synthesized; αGT-1(5'GAATTCAAGCTTATGATCACTATGCTTCAAG-3') which in the sense oligonucleotide encoding the first six amino acids of the mature αGT and contains a HindIII restriction site, and αGT-2(5'GAATTCCTGCAGTCAGACATTATTCTAAC-3') which is the anti-sense oligonucleotide encoding the last 5 amino acids of the mature αGT and the in phase termination codon and contains a PstI restriction site. This oligonucleotide pair was used to amplify a 1185 bp fragment from a C57BL/6 spleen cell cDNA library (Sandrin et al (1992)

J Immunol. 194:1636-1641). The 1185 bp fragment was purified from a Low Gelling point agarose gel, digested with HindIII and PstI (Pharmacia) restriction endonucleases, and directionally cloned into HindIII/PstI digested CDM8 vector (Seed B (1987) Nature 329:840-842) using T4 ligase (Pharmacia). The product of the ligation was used to transform MC1061/p3, and DNA prepared from resultant colonies for further examination. One plasmid (pαGT-3) having the 1185 bp fragment was selected for further studies. Plasmid DNA was prepared, sequenced to confirm the correct DNA sequence, and used for COS cells transfection experiments using DEAE/Dextran (Vaughan et al (1991) Immunogenetics 33:113-117; Sandrin et al (1992) J Immunol. 194:1636-1641; Seed B (1987) Nature 329:840-842).

EXAMPLE 2

Human anti-pig antibodies detects epitopes present on different cells. To establish that human serum contains antibodies to pig cells which are predominantly of the IgM class, a pool of human serum was made (from 10 donors) and found to contain antibodies which reacted with pig red cells (by hemagglutination); pig lymphocytes (rosetting assay and flow cytometry); pig spleen cells (rosetting); and pig endothelial cells (flow cytometry) (Figures 1 and 2). Absorption studies emonstrated that the same xeno antigens were present on all of these tissues (Figure 1 and Figure 2), as absorption with RBC, spleen cells or PBL, removed reactivitiy for the other cells (Figure 1A and Figure 2. absorption of the serum pool with EC, while removing all of the EC reactive antibodies (Figure 2a), completely removed all PBL reactive antibodies and almost all the RBC hemagglutinating antibodies (titer fell from 1/128 to 1/2) (Figure 1A). Absorption with RBC removed 75% (Figure 2b) and spleen cells all (Figure 2c) of the EC reactive antibodies shown by flow cytometry. Thus, common epitopes are present on pig red cells, PBL< spleen and endothelial cells.

Most of the activity in the serum pool was due to IgM rather than IgG antibodies as indicated by the inability of a protein A-sepharose column, which does not bind IgM, to alter

the titer of the serum after passage through the column (Figure 3), and IgG antibodies eluted from the protein A-sepharose column reacted only weakly with RBC (Figure 3). Furthermore, treatment of the serum with 2-mercaptoethanol, which destroys IgM but leaves IgG intact, led to a complete loss of antibody activity (Figure 3). When the serum was fractionated by sephacryl gel chromatography, the high molecular weight fractions (IgM) were reactive with RBC, whereas the low molecular weight fractions (IgG) were not (data not shown). Thus the different pig cells carry similar epitopes, all reacted with IgM antibodies and in our assays there was little IgG activity found in the human serum for pig cells.

EXAMPLE 3

Human anti-pig antibodies react predominantly with terminal galactose residues. The ability of different carbohydrates to inhibit the hemagglutination reaction (Figure 4) was examined. Of the sugars tested, inhibition as measured by a decrease in titer, was observed with 300mM galactose, methyl-α-D-galactopyranoside, melibiose and stachyose, all of which decreased the titer of the serum pool by 75% (Figure 4); and with 300mM D-galactosamine, for which a 50% decrease in titer was observed (Figure 4). None of the other monosaccharides tested (listed in the figure legend) had any effect on hemagglutination titer (Figure 4). These studies demonstrated that galactose is the part of the epitope, as both melibiose and stachyose have terminal galactose residues. It is of intrest to note the difference in the ability of galactose in the α(methyl-α-D-galactopyranoside, melibiose and stachyose) but not β(methyl-β-D-galactopyranoside) configuration to inhibit the serum.

The relative avidity of the antibodies for the sugars which inhibited agglutination was estimated from the concentration of sugar giving 50% inhibition of the agglutination titer (Figure 5). Both D-galactose and melibiose achieved this inhibition at <1.5mM, stachyose and methyl-α-D-galactopyranoside at 4.7mM and D-galactosamine at 18.7mM (Figure 5). By contrast, D-glucose and methyl-β-D-galactopyranoside had no effect even at 300mM

concentration. Thus D-galactose is an important part of the epitope, as it is a potent inhibitor of the xenoantibodies at low concentration (<1.15mM); the ability of methyl-α-D-galactopyranoside to inhibit agglutination at low concentrations (<1.15mM), compared with the failure of methyl-β-D-galactopyranoside (300mM) to inhibit, demonstrates that the galactose residue (which is likely to be a terminal sugar) is in an α-linkage rather than a β-linkage with the subterminal residue. The results obtained with melibiose (Galα(1,6)Gal) and stachyose (Galα(1,6)Galα(1,6)Glcβ(1,2) Fru), which have α-linked terminal galactose residues, are in accord with this conclusion. The inhibition of hemagglutination observed with galactosamine, which has an additional amine side chain on galactose, (50% inhibition of titre at 18.7mM) could be due to a second carbohydrate involved in the epitope, or a lower affinity of the xenoantibodies for this sugar.

To further examine the reaction with galactose, the serum pool was absorbed four times with equal volumes of packed melibiose sepharose or with sepharose as the control (Figure 6), one absorption with melibiose-sepharose decreased the titer of the antibody from 1/32 to 1/4, and two sequential absorptions decreased the titer further to 1/2 (Figure 6). This absorption was specific for melibiose, as using sepharose beads had no effect (Figure 6). Thus the majority of the antibody (=94%) reactive with xenoantigens reacts with galactose in an α-linkage.

EXAMPLE 4

Human anti-pig antibodies react with of COS cells after transfection with α(1,3)galactosyl transferase. The cDNA coding for the α(1,3)galactosyl transferase which transfers a terminal galactose residue with an α(1,3) linkage to a subterminal galactose has been cloned for both mouse (Larsen et al (1989) J Biol Chem 264:14290-14297) and ox (Joziasse et al (1989) J Biol Chem 264:14290-14297). Using this data we used transfection experiments to determine the role of the Galα(1,3)Gal epitope in isolation of others. The

mouse transferase was isolated from a cDNA library using the PCR technique, and the PCR product was directionally cloned into the CDM8 vector for expression studies in COS cells. The cDNA insert was sequenced in both directions and shown to be identical to the published nucleotide sequence (Larsen et al (1989) J Biol Chem 264:14290-14297). COS cells, derived from Old Word Monkeys, were chosen as they do not react with human serum nor with the IB-4 lectin (which is specific for the Galα(1,3)Gal epitope) (Table 1). After transfection of COS cell with the α(1,3)galactosyl transferase, the Galα(1,3)Gal epitope was detected on the cell surface by binding of the IB-4 lectin (Table 1); these cells were also strongly reactive with the serum pool. Absorbing the human sera with pig RBC removed the reactivity for Galα(1,3)Gal$^+$COS cells, (Table 1). Passage of the serum over a protein-A sepharose column had no effect on the reactivity of the serum for Galα(1,3)Gal$^+$COS cells, when using a FITC conjugated sheep anti-human IgM as the second antibody (this was reflected in the same number of reactive cells, the intensity of staining and the titer of the serum (Table 1)). In contrast to this, eluted antibodies reacted only weakly with the Galα(1,3)Gal$^+$COS cells, and this reaction was only observed when using FITC conjugated sheep anti-human IgG or FITC conjugated sheep anti-human Ig, but not FITC conjugated sheep anti human IgM (Table 1). Thus human serum has IgM antibodies to the Galα(1,3)Gal epitope which was expressed on Galα(1,3)Gal$^+$COS cells. The reaction of the serum with Galα(1,3)Gal$^+$COS cells is specific and not due to the transfection procedure as CD48$^+$ COS cells were not reactive with either the serum nor the IB-4 lectin (Table 1). Furthermore, the reactivity for both pig RBC (as detected by hemagglutination) and EC (as detected by FACS analysis) could be removed by absorption with Galα(1,3)Gal$^+$COS cells but not untransfected COS cells. Thus human serum pool contains IgM antibodies reactive with the Galα(1,3)Gal epitope.

EXAMPLE 5

Cloning of porcine α(1,3)galactosyl transferase. Utilizing the murine cDNA clone for the α(1,3)galactosyl transferase as a hybridisation probe we have cloned the pig

$\alpha(1,3)$galactosyl transferase from a pig cDNA library according to standard methods as described in Sambrook et al (Supra). Figure 7 shows the nucleotide seuqence and predicted amino acid sequence of pig Gal$\alpha(1,3)$ transferase. The sequence shown is incomplete at the 5' end. The pig transferase has high sequence homology with both the murine and bovine $\alpha(1,3)$galactosyl transferase genes.

The finding that the majority of xenoreactive IgM is directed to the enzymatic product of the single transferase raises the possibility of producing transgenic pigs lacking the epitope, by targetted destruction of the $\alpha(1,3)$galactosyl transferase genes using homologous recombination technology. Such genetically modified pigs could be used for transplantation. The destruction of the gene is likely to have no deleterious effect on the pig - humans live normally in its absence.

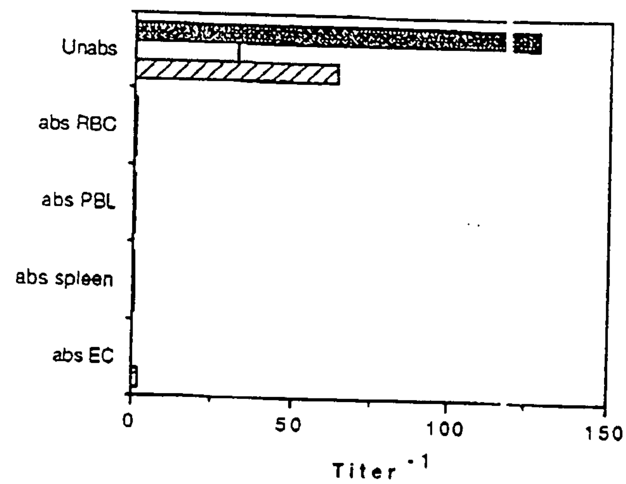
This invention has been described by way of example only and is in no way limited by the specific examples herewith.

DATED this 16th day of March 1993,

THE AUSTIN RESEARCH INSTITUTE
By Its Patent Attorney
DAVIES COLLISON CAVE

FIGURE 1/7

A



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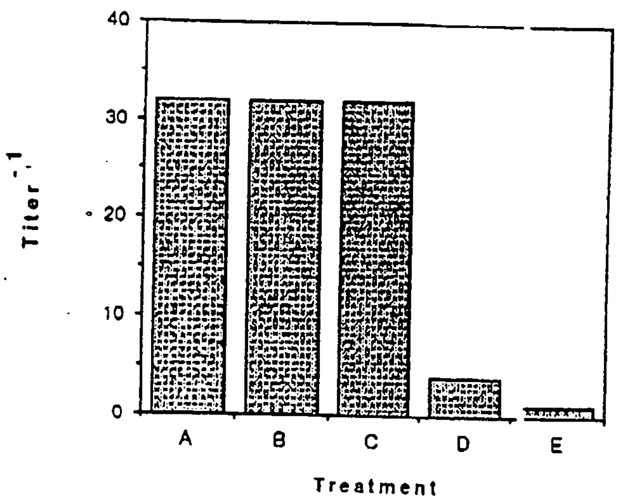
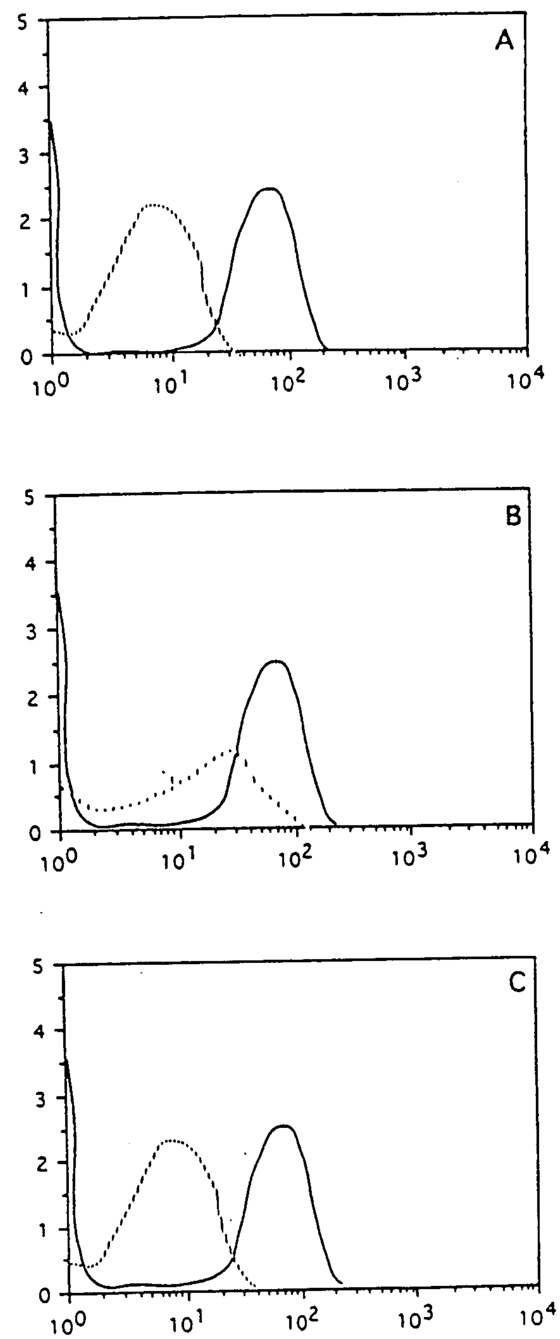


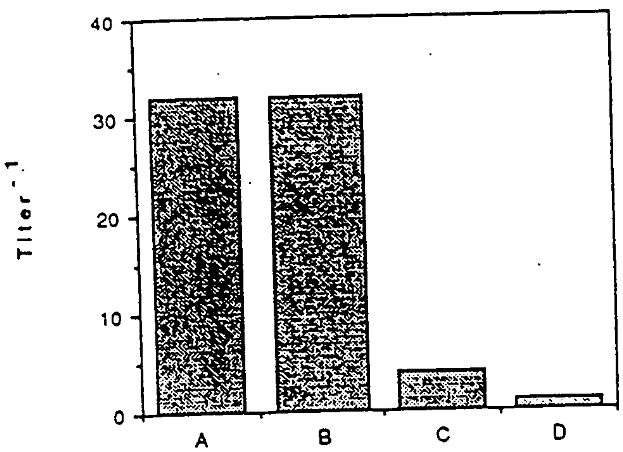
AUSTIN1.SPEC

FIGURE 2/7



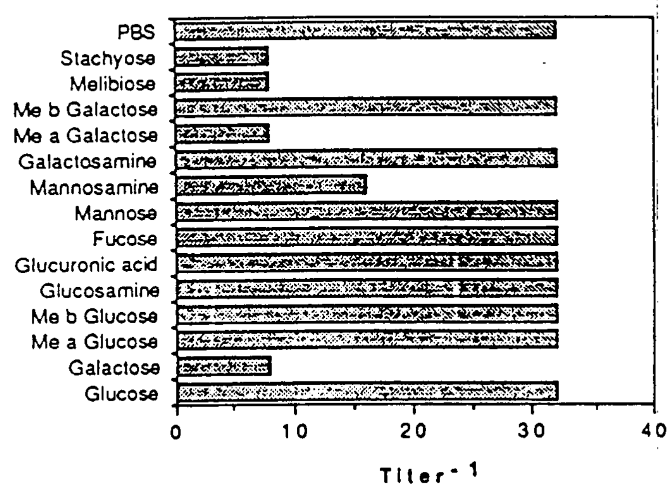
AUSTIN1.SPEC

FIGURE 3/7



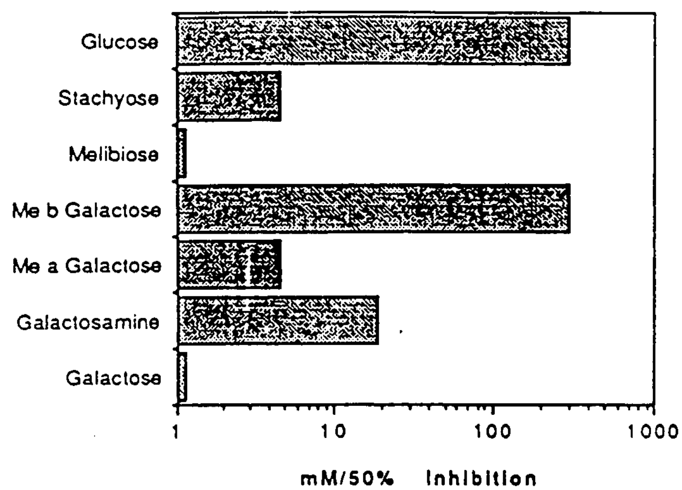
AUSTIN1.SPEC

FIGURE 4/7



AUSTIN1.SPEC

FIGURE 5/7



AUSTIN1.SPEC

FIGURE 6/7

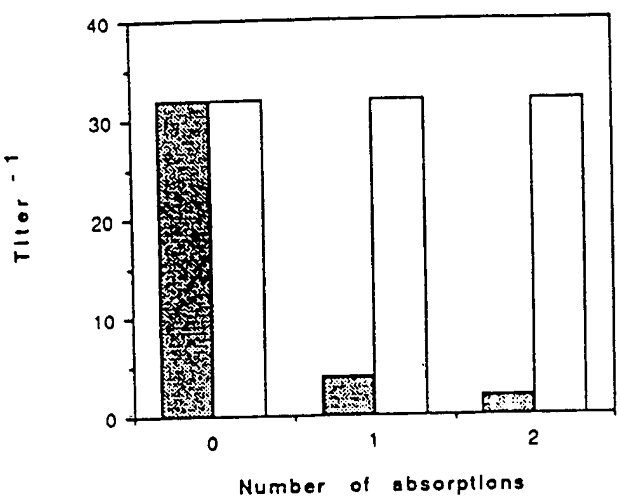


FIGURE 7/7

Nucleotide and predicted amino acid sequence of pig Gal α(1,3) Gal Transferase

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val pro ser ser asn ser ala ser gln ser pro gln ala met thr asp pro cys ser pro
GTA CCG AGC TCG AAT TCC GCA ACC CAG TCA CCA CAA GCC ATG ACT GAC CCA TGT TCC CCC     60

arg leu ser tyr leu ser lys ala ile leu thr leu cys phe val thr arg lys pro pro
AGA CTG TCG TAC CTT AGC AAA GCC ATC CTG ACT CTA TGT TTT GTC ACC AGG AAA CCC CCA    120

glu val val thr ile thr arg trp lys ala pro val val trp glu gly thr tyr asn arg
GAG GTC GTG ACC ATA ACC AGA TGG AAG GCT CCA GTG GTA TGG GAA GGC ACT TAC AAC AGA    180

ala val leu asp asn tyr tyr ala lys gln lys ile thr val gly leu thr val phe ala
GCC GTC TTA GAT AAT TAT TAT GCC AAA CAG AAA ATT ACC GTG GGC TTG ACG GTT TTT GCT    240

val gly arg tyr ile glu his tyr leu glu glu phe leu ile ser ala asn thr tyr phe
GTC GGA AGA TAC ATT GAG CAT TAC TTG GAG GAG TTC TTA ATA TCT GCA AAT ACA TAC TTC    300

met val gly his lys val ile phe tyr ile met val asp asp ile ser arg met pro leu
ATG GTT GGC CAC AAA GTC ATC TTT TAC ATC ATG GTG GAC GAT ATC TCC AGG ATG CCT TTG    360

ile glu leu gly pro leu arg ser phe lys val phe glu ile lys ser glu lys arg trp
ATA GAG CTG GGT CCT CTG CGT TCC TTT AAA CTG TTT GAG ATC AAG TCC GAG AAG AGG TGG    420

gln asp ile ser met met arg met lys thr ile gly glu his ile leu ala his ile gln
CAA GAC ATC ACC ATG ATG CGC ATG AAG ACC ATC GGG GAG CAC ATC CTG GCC CAC ATC CAG    480

his glu val asp phe leu phe cys ile asp val asp gln val phe gln asn asn phe gly
CAC GAG GTG GAC TTC CTC TTC TGC ATT GAC GTG GAT CAG GTC TTC CAA AAC AAC TTT GGG    540

val glu thr leu gly gln ser val ala gln leu gln ala trp trp tyr lys ala his pro
GTG GAG ACC CTG GGC CAG TCG GTC GCT CAG CTA CAG GCC TGG TGG TAC AAG GCA CAT CCT    600

asp glu phe thr tyr glu arg pro lys glu ser ala ala tyr ile pro phe arg gln gly
GAC GAG TTC ACC TAC GAG CGG CCG AAG GAG TCC GCA GCC TAC ATT CCG TTT CGC CAG GGG    660

asp phe tyr tyr his ala ala ile leu gly gly thr pro thr gln val leu asn ile thr
GAT TTT TAT TAC CAC GCA GCC ATT TTG GGG GGA ACA CCC ACT CAG GTT CTA AAC ATC ACT    720

gln glu cys phe lys gly ile leu gln asp lys glu asn asp ile glu ala glu trp his
CAG GAG TGC TTC AAG GGA ATC CTC CAG GAC AAG GAA AAT GAC ATA GAA GCC GAG TGG CAT    780

asp glu ser gly leu asn lys tyr phe leu leu asn lys pro thr lys ile leu ser pro
CAT GAA AGC GGG CTA AAC AAG TAT TTC CTT CTC AAC AAA CCC ACT AAA ATC TTA TCC CCA    840

glu tyr cys trp asp tyr his ile gly met ser val asp ile arg ile val lys gly ala
GAA TAC TGC TGG GAT TAT CAT ATA GGC ATG TCT GTG GAT ATT AGG ATT GTC AAG GGG GCT    900

trp gln lys lys glu tyr asn leu val arg asn asn ile ***
TGG CAG AAA AAA GAG TAT AAT TTG GTT AGA AAT AAC ATC TGA CTT TAA ATT GTG CCA GCA    960

GTT TTC TGA ATT TGA AAG AGT ATT ACT CTG GCT ACT TCC TCA GAG AAG TAG CAC TTA ATT   1020

TTA ACT TTT CAA AAA ATA CTA ACA AAA TAC CAA CAC AGT AAG TAC ATA TTA TTC TTC CTT   1080

CCA ACT TTG AGC CTT GTC AAA TGG GAG AAT GAC TCT GTA GTA ATC AGA TGT AAA TTC CCA   1140

ATG ATT TCT TAT CTG CGG AAT TCC AGC TGA GCG CCG GTC CTA CCA TTA CCA GTT GGT CTG   1200

GTG TCG ACG ACT CCT GGA GCC CGT CAG TAT CGG CGG AAT TCG CGG CCG GGC GCC AAT GCA   1260

TTG GGC CCA ATT CCG CCC TAT AGT GAG TCG TAT TAC AAT TCA CTG GCC GTG TTT TAC AAC   1320

CTC GTG ACT GGG AAA ACC CTG GCC TTA CCC AAC                                       1353
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